

## **APPENDIX A**

### **VENDOR'S CLAIMS**

This appendix was generated and written solely by Envirogen. The statements presented herein represent the vendor's point of view and summarize the claims made by the vendor, Envirogen (Lawrenceville, New Jersey), regarding their in-situ propane biostimulation technology. Publication herein does not represent the EPA's approval or endorsement of the statements made in this section; the EPA's point of view is discussed in the body of this report.

#### **A.1 Introduction**

MTBE has been used extensively as a gasoline additive in the United States to enhance combustion efficiency and reduced vehicle emissions, and its widespread use has ultimately led to its accidental release in the environment. Because it is present in high concentrations in reformulated gasoline and highly soluble in groundwater ( $K_{ow}$  1.05), even small releases of gasoline can result in large MTBE plumes. The incidence of spills of MTBE-containing fuels from confirmed leaking underground storage tanks (USTs) in the United States has been estimated to be as high as 250,000. Sites contaminated with MTBE can vary in size from large terminals owned by multinational corporations to small family-owned service stations located near residential neighborhoods. Remedial technologies for treating MTBE, therefore, must be efficient, cost effective, and adaptable to a wide range of site conditions and limitations. Traditional remedial technologies such as activated carbon adsorption and air-sparging have proven to be largely ineffective or expensive for treating MTBE contamination, and it is clear that no single technology is suitable for every contaminated site. Recently, bioremediation has emerged as a suitable remedial alternative for some sites, and it can be applied by stimulating indigenous MTBE-degrading bacteria, or by adding exogenous bacteria, depending on conditions at the target site.

#### **A.2 Biostimulation Technology Description**

Biostimulation is a process by which the degradative activity of indigenous or added microorganisms is enhanced by adding specific nutrients or co-substrates that might otherwise be lacking or limiting. Often, indigenous microbes can be stimulated simply by adding a missing terminal electron acceptor like oxygen. Because some contaminants are not good growth substrates for indigenous bacteria, biostimulation sometimes can be facilitated by adding a co-metabolic growth substrates. Co-metabolism

is a process by which the same enzyme that degrades a good growth substrate also fortuitously degrades the contaminant, often with little or no benefit to the degradative organisms. We demonstrated that propane oxidizing bacteria can co-metabolically mineralize MTBE to CO<sub>2</sub> and H<sub>2</sub>O after growth on propane (Steffan et al., 1997). Because other hydrocarbon gases, such as methane and butane, have been used to stimulate co-metabolic biodegradation processes in situ, it is likely that a similar application of biostimulation, whereby propane and oxygen are injected to stimulate MTBE degradation by indigenous organisms or seed cultures, is feasible at some sites (US Patent # 5,814,514, Sept. 29, 1998).

There are several potential advantages to using a co-metabolic biostimulation approach for degrading MTBE in situ. Co-metabolism uncouples biodegradation of the contaminant from growth of the organisms. That is, the microbes can be supplied sufficient co-substrate (e.g., propane) to support growth, so they do not have to rely on the utilization of low levels of contaminants to maintain their survival. Also, the technology can be applied in a number of configurations depending on site characteristics and treatment needs. Possible application scenarios include: 1) re-engineered or modified multi-point AS/SVE systems that deliver propane and air throughout a contaminated site (suitable for use with existing AS/SVE systems or specially designed systems); 2) a series of air/propane delivery points arranged to form a permeable treatment wall to prevent off site migration of MTBE; 3) permeable treatment trenches fitted with air and propane injection systems; 4) in situ recirculating treatment cells that rely on pumping and reinjection to capture and treat a migrating contaminant plume; and 5) propane and oxygen injection through bubble-free gas injection devices to minimize off-gas release and contaminant stripping. Furthermore, propane is widely available, transportable even to remote sites, already present at many gasoline stations, and relatively inexpensive. Thus, propane biostimulation has the potential to be an attractive remediation option at a wide variety of MTBE-contaminated sites.

### **A.3 Demonstration results**

During this project, we applied and evaluated propane biostimulation for MTBE remediation at the Port Hueneme, CA National Environmental Technologies Test Site. The primary purposes of this field demonstration included:

- Evaluating the effectiveness of propane biostimulation for MTBE remediation
- Optimizing sparging and SVE flow rates and injection/extraction cycles;
- Quantitatively assessing the impact of propane sparging on soil gas and ambient air quality;
- Delineating the zone of influence of the treatment;

- Assessing the potential for subsurface gas migration and fugitive emissions; and,
- Assess our ability to degrade MTBE to less than 5 µg/L with a single row of propane and oxygen injection points.

Microcosm testing with samples from the site revealed that the resident groundwater had low indigenous MTBE degrading microbial activity, even though MTBE degradation by native organisms has been observed during other demonstrations near our test plots. Consequently, we elected to seed our Test plot with a seed culture of propane oxidizing bacteria to initiate biodegradation

During the demonstration MTBE was degraded in both our Test (propane, oxygen, and bacteria added) and Control Plot (no propane added), but in neither case were the MTBE concentrations maintained at below the desired level of 5 µg/L. However, low levels of MTBE were achieved in many of the monitoring wells. For example, MTBE concentrations in the first row of deep Test Plot monitoring wells, GWT-2D, GWT-3D, and GWT-4D, went from 850, 1440, and 1440 µg/L at the beginning of the treatment (6/12/01) to 19, 46, and 440 µg/L at the end of treatment (3/12/02), respectively. Mean MTBE concentrations in the second row of monitoring wells went from 1967 µg/L (+/- 556 µg/L; n=3) to 148 µg/L (+/- 88 µg/L; n=3) during the same period. Likewise, MTBE concentrations of <5 µg/L were achieved in at least two of the shallow monitoring wells in the test plot. These low levels were achieved despite the addition of dMTBE as a tracer by the EPA which increased the total load of MTBE to the test plots. Variability in groundwater flow through the plots, and temporally during the course of the demonstration, appeared to affect distribution of co-substrates and oxygen in the test plot, and it made it difficult to accurately quantify the extent of MTBE degradation in the plots.

At the end of the field demonstration, experiments were performed to isolate MTBE degrading organisms from both the Test and Control Plot. Enrichment culturing with propane as a carbon source allowed growth of propane/MTBE degrading microorganisms from the Test Plot, but not from the Control Plot. Isolated propanotrophs from the Test Plot were phenotypically different (colony morphology and color) than the *Rhodococcus ruber* ENV425 culture added to the aquifer. Organisms able to grow on MTBE as a sole carbon source were isolated from both plots. These results suggest that the addition of propane to the Test Plot did allow growth of indigenous propane oxidizing microorganisms that were able to degrade MTBE. Similarly, addition of oxygen to both plots appeared to stimulate the growth of indigenous microbes capable of growth on MTBE.

Response to oxygen addition in the Control Plot was more rapid than anticipated based on microcosm studies performed by us, and based on prior demonstrations at the site. This high level of activity frustrated analysis of the effect of propane biostimulation on MTBE degradation at the site. Likewise, changes in the groundwater flow also made analysis of the data difficult. For example, because degradation rate calculations are dependent on groundwater flow, and because the hydraulic gradient was flat and the flow was low at the site, even small variations in flow could significantly affect degradation rate calculations. Groundwater elevation data even suggested that groundwater flow may have reversed its flow direction periodically during the study, especially in the Test Plot. Thus, unlike our prior demonstration where the positive effects of propane biostimulation were obvious (see below) the effects are less apparent in results of this study.

This demonstration also demonstrated that propane biosparging can be safely and economically applied at the field scale. Application of the technology resulted in no measurable fugitive emissions of propane, and in situ biodegradation and controlled propane addition maintained propane levels near or below its detection limit in groundwater. Propane costs for the 10-month demonstration were only about \$50/month, indicating that application of this technology costs little more than a traditional air sparging system. Because of low propane emissions, the technology should not require secondary containment systems (e.g., soil vapor extraction) in most cases. Thus, it may be cost effective to incorporate propane biosparging equipment into MTBE remediation designs, even at sites where MTBE biodegradation by indigenous organisms is suspected. If indigenous bacteria prove to be inefficient or ineffective at remediating the site, propane can be injected to enhance activity at minimal additional cost.

Results of this demonstration also suggested that most of the active MTBE degradation that occurred in both plots occurred near the oxygen injection points. Thus, degradation activity may have been limited by the availability of oxygen in the subsurface. Oxygen was likely consumed by both geochemical oxygen sinks and biological activity. Because of the process monitoring and technology validation procedures of both Envirogen and the EPA, we elected not to increase gas flows into the site during this demonstration. To reach even lower MTBE levels, however, either additional rows of oxygen injection points or higher oxygen loading rates may be needed.

#### **A.4 Case Study**

**Introduction.** Propane biostimulation for MTBE remediation was applied at an operating Camden County, New Jersey service station site. A site investigation was initiated at the site after one of the site's

underground gasoline tanks failed a tightness test in July 1988. The site has since undergone a range of remedial actions including soil excavation and air sparging. Six on-site groundwater monitoring wells (MW-5 to MW-10) and two offsite wells (MW-11 and MW-12) were installed to monitor BTEX and MTBE (Figure A-1). These wells are currently being monitored on a quarterly basis. Groundwater samples collected on February 9, 1999 showed site MTBE concentrations ranging from 170 µg/L (at upgradient monitoring well MW-8) to 270,000 µg/L (MW-6). Historical groundwater MTBE data from 1990 to 1999 indicate increasing concentrations at monitoring wells MW-6, MW-7, MW-9 and MW-11.

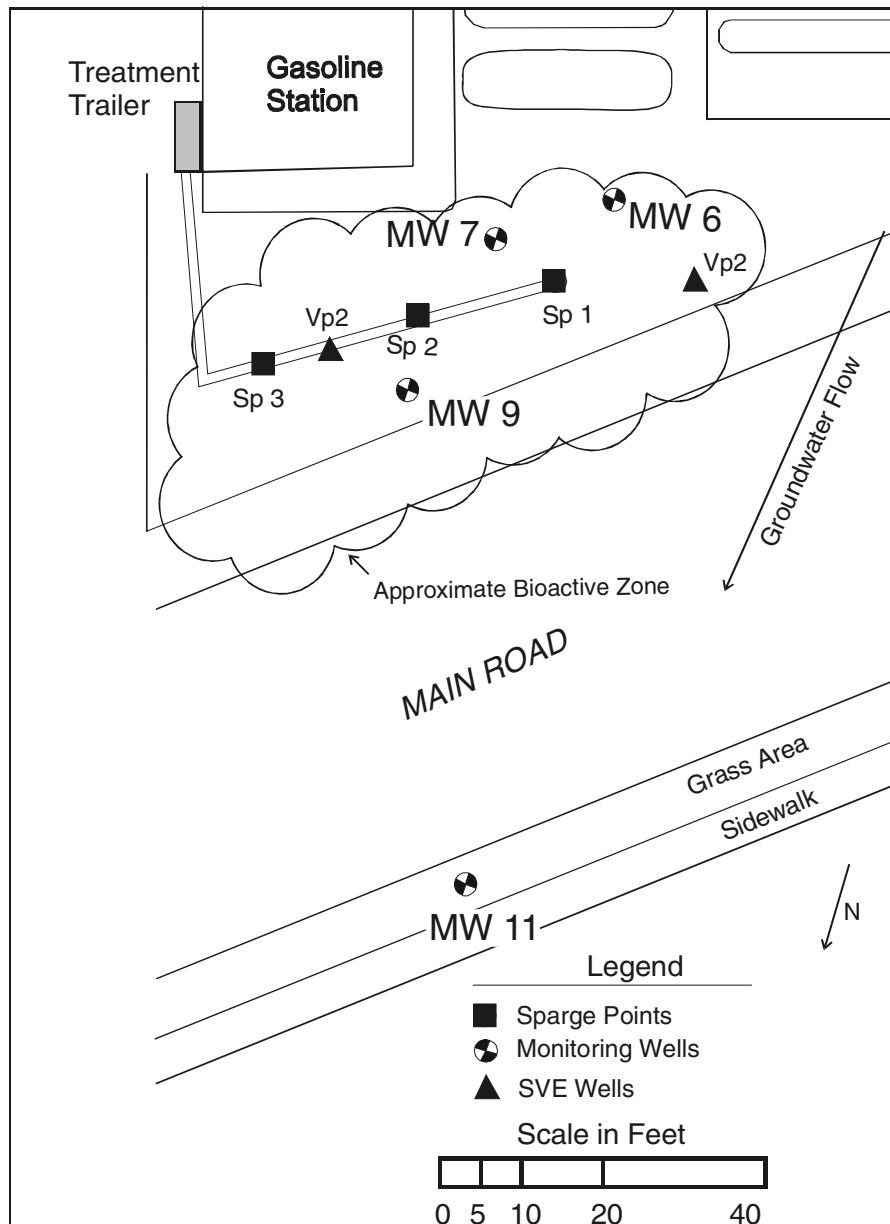
Because of the failure of air sparging and soil vapor extraction to sufficiently remove MTBE from the site groundwater, Envirogen was asked to perform propane biostimulation at the site. A biosparging and propane injection system was designed to allow flexible and safe implementation in the field. The system consisted of injection and SVE components, and utilize existing sparge wells (SP-1, SP-2 and SP-3) and SVE wells (VP-1, VP-2 and MW-10) at the site. The injection system consisted of two separate components; an air compressor and a propane supply system that was connected to the existing sparging distribution lines via a common manifold. An in-line filter was installed on the injection line to remove moisture and/or oil escaping the air compressor. The SVE system consisted of a vacuum blower that was connected to the existing SVE distribution lines and a carbon canister for treatment of the off-gas. Operation of the system was controlled using a common control panel with redundant control switches to ensure safe operations. An interlock device was used to prevent propane injection unless the SVE system was operational.

Because the existing air sparging wells were not designed and constructed for pulsed operation, operation of the wells in a pulsed mode resulted in an accumulation of silt in the wells and reduced airflow. Consequently, the sparging system was operated with a continuous low airflow of 13 scfm. A 10-pound propane gas cylinder (e.g., similar in size to home barbecue propane tanks) was used as the propane supply. The discharge from the propane cylinder was controlled by a flow valve and pressure indicator mounted on the cylinder. A pressure control valve set at 40 psi was utilized to monitor and control the propane pressure in the line. An in-line propane lower explosive limit (LEL) detector was installed to continuously monitor the LEL level and ensure safe operation of the system. Dedicated flow meters were installed on each line to control the flow to each sparge well. Propane was added to the air stream for 10 min every three hours at a rate that ensured that the propane concentration did not exceed 0.2% propane in air (10% of the propane LEL). Approximately 0.5 lbs. of propane and 315 lbs. of oxygen were added to the site each day.

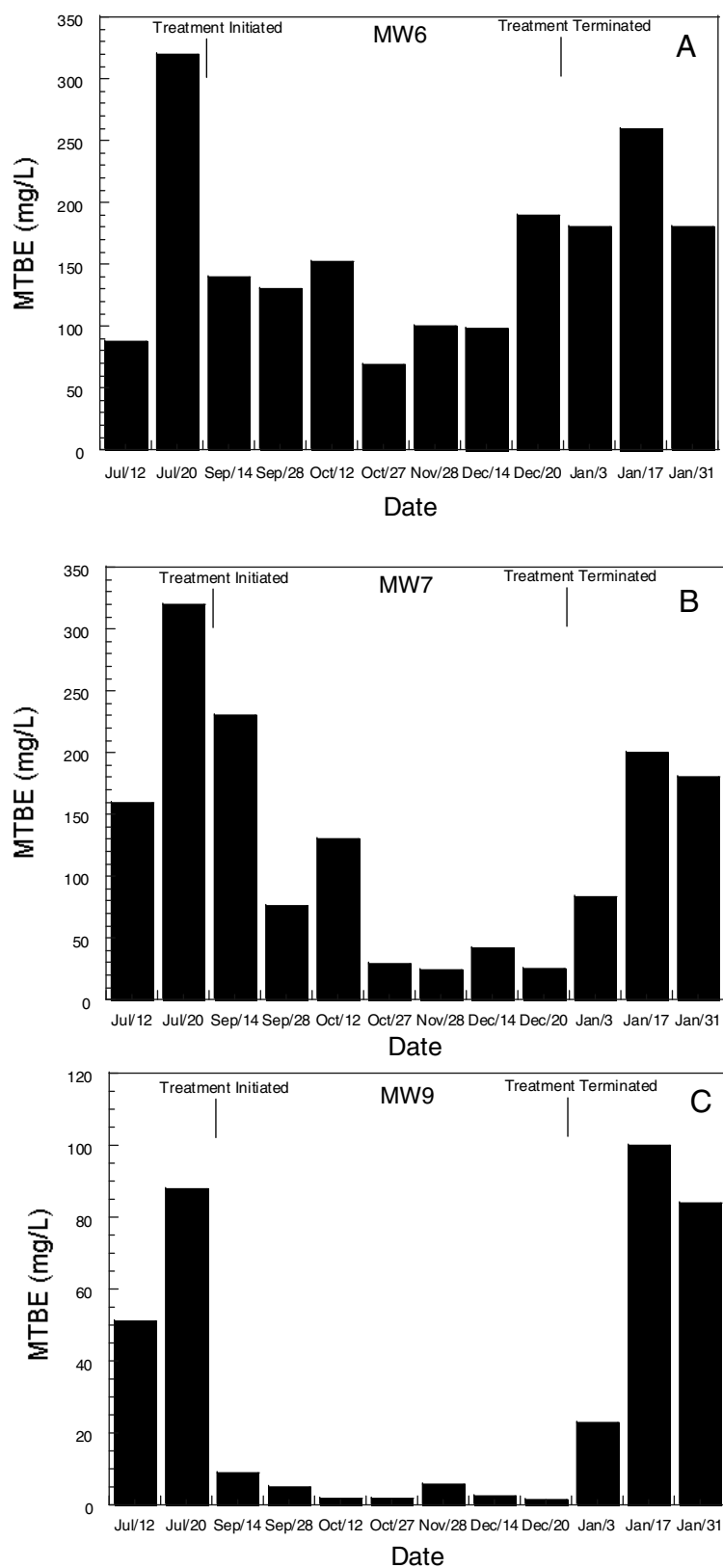
**Results.** Preliminary laboratory studies revealed that the site had low levels of indigenous microorganisms, presumably because of the low groundwater pH (pH ~3.5 to 5). Therefore, we elected to seed the aquifer with *R. ruber* ENV425. The system was initially operated for approximately one month without the addition MTBE degrading microorganisms. A total of 17 L of culture of strain ENV425 ( $\sim 1 \times 10^{11}$  cells/ml) was then added to the three sparge points. Bacterial injection was followed by several cycles of air sparging to help distribute the microbes into the treatment zone, and two days of continuous propane and air sparging to aid in establishing an active MTBE degrading microbial population. Because the low measured pH in ground water at the site, the ground water needed to be buffered to raise the pH to a range more favorable to MTBE biodegradation. A buffer solution of sodium bicarbonate was added to the sparge point periodically during the demonstration to achieve this goal. During each buffering event, a total of 120 gallons of a sodium bicarbonate solution was added to the sparge points followed immediately by air sparging to disperse the buffer into the formation. The system was operated for an additional ~5 months before a scheduled shutdown. MTBE and BTEX concentrations in the groundwater were measured using EPA method 8260.

Groundwater monitoring during the project was performed in monitoring wells MW-6, MW-7, MW-9, and MW-11 (Figure A-1). MW-6 is located just upgradient of the treatment zone, but it was slightly influenced by the treatment as indicated by increased dissolved oxygen in the groundwater during the treatment system operation. MW-7 also was upgradient of the treatment wells, but clearly within the zone of influence of the propane and oxygen injection system. MW-9 was immediately down gradient of the sparging points, and MW-11 was far down gradient of the treatment system.

MTBE concentrations in MW-6 were reduced by approximately 40% during the 5-month treatment period (Figure A-2A). Likewise, MTBE concentration in MW-7 were reduced by as much as 76% during biostimulation treatment (Figure A-2B). MTBE concentrations in MW-9 were reduced by as much as 98%, from 88 mg/L to 1.7 mg/L, during the treatment period (Figure A-2C). MTBE concentrations in MW 11 were relatively constant during the 5-month treatment period (data not shown), presumably because it was too far down gradient for treated water did not reach it during the demonstration period. First order rate constants for MW-6, MW-7 and MW-9 were calculated to be 0.0084, 0.0288, and 0.0027/day, respectively.



**Figure A-1.** Field site and system layout. Propane and air were injected into three existing air sparging points (Sp1, Sp2, and Sp3), and MTBE concentrations were measured in MW6, MW7, MW9, and MW11.



**Figure A-2.** MTBE concentrations in groundwater at on-site monitoring wells at a Camden County, New Jersey service station before, during, and after propane biostimulation treatment.

This corresponded to MTBE half-lives of 82, 24, and 30 days, respectively. After nearly 5 months of operation the treatment system was shut down. In each of the treatment zone monitoring wells the MTBE concentration rebounded to near pre-treatment levels (see Figure A-2A-C). The rebound effect was attributed to a continuing source of MTBE contamination at the site. Ongoing work at the site has led to a repair of the leakage source and implementation of an expanded treatment system for full-scale remediation of the site, including the source area.

TBA concentrations in the site groundwater increased during MTBE biodegradation, but they were typically several orders of magnitude lower than MTBE concentrations. During our initial work with propane oxidizing bacteria, pure cultures produced nearly stoichiometric concentrations of TBA from MTBE (Steffan et al., 1997). TBA concentrations in the cultures decreased only after MTBE was completely degraded. At this site, however, TBA was apparently degraded simultaneously with MTBE because it did not accumulate to levels near the initial MTBE concentration. Furthermore, TBA concentrations declined rapidly after propane injection was terminated and MTBE degradation ceased. The decline in TBA concentrations was accompanied by a decline in oxygen concentration. These data suggest that the propane oxidizers continued to degrade TBA after propane was no longer available to induce MTBE degradation, or that other TBA degraders were present in the system. During microcosm studies with ENV425 the organisms degraded TBA to <5 µg/L, indicating that similar levels will be achieved in the field provided the treatment period is sufficiently long.

The results of the case study showed that MTBE-contaminated groundwater can be biologically remediated using propane oxidizing bacteria and propane biosparging. This site presented a number of unique challenges to this technology, including low pH, high MTBE concentrations, and a continuing source of MTBE. Nonetheless, a significant mass of MTBE was removed during this demonstration, and MTBE reductions of greater than 90% were achieved in a relatively short time. The results also suggest that this treatment approach also supports the degradation of TBA.

Propane proved to be an excellent substrate for biostimulation applications; it is widely available, transportable even to remote sites, and relatively inexpensive. Application of propane injection in the field, however, may raise concerns about creating explosive mixtures of propane and air in situ. To address these concerns we injected propane in pulses and did not exceed 10% of the LEL of propane in the injection gas. We also used SVE to prevent in situ accumulation of propane. The results of our monitoring, however, suggest that propane stripping is minimal and SVE is likely unnecessary at most sites.

**Technology Costs.** Estimates of the cost of implementing the propane biostimulation system are similar to the costs of applying conventional air sparging/biosparging at a service station site. During the case study, propane costs were only \$240 for the entire 6 months of operation. The primary equipment cost for the application is a biosparging system that safely blends low levels of propane with sparging air. A typical system, fully engineered, constructed and mounted in a trailer is expected to cost approximately \$35,000, but the mobile system is suitable for repeated use at multiple sites, or it could be returned to a site to remediate future MTBE releases. Stationary systems can be installed at a lower cost. Based on the results of the project, future applications of the technology probably will not require the use of SVE during biosparging, saving both the equipment and discharge permit costs. It also is recommended that pre-design treatability studies be performed with site groundwater and soil. These tests are expected to cost ~ \$4,000. Addition of seed cultures, when needed, is expected to cost ~\$1000 to \$2000 per application depending on the size of the site. The technology also can be applied in a number of alternative configurations — some employing existing systems — depending on site characteristics and treatment needs. Thus, the complexity of the site and the selection of an application design will ultimately determine the total cost of the system.

## A.5 Summary

Propane biostimulation is a useful and economical in situ treatment alternative for remediating MTBE contaminated groundwaters. The technology is very flexible and can be combined with other traditional technologies like air sparging and soil vapor extraction to enhance the removal of MTBE from groundwater. Importantly, the technology also promotes the removal of TBA from groundwater. Because TBA is highly water soluble and not easily removed by air sparging, soil vapor extraction, or carbon adsorption, the ability to simultaneously remove MTBE and TBA in a single treatment process, and in situ, should present a considerable cost savings to users of the technology. Demonstrations performed to date show that the technology can be applied safely with little risk of fugitive propane emissions or accumulation in the subsurface.

Propane biostimulation should be considered as a remedial alternative for sites where air sparging or the addition of oxygen alone does not support MTBE remediation (see Case Study above). Likewise, it should be considered in regions of the country where TBA in groundwater also is tightly regulated. Furthermore, the potential application of propane biostimulation should be considered when installing an air sparging system at an MTBE contaminated site. By creating a flexible system that will allow the subsequent application of propane injection in the event that air sparging alone is not sufficient, considerable cost savings can potentially be realized in overall treatment costs. Similarly, the subsequent addition of propane for in situ biostimulation should be considered when planning the use of other technologies such as cut-off trenches and bioaugmentation with MTBE degrading microbes. In all cases, it is recommended that treatability studies be performed prior to designing and implementing propane biostimulation systems. Treatability studies can provide information about the availability of indigenous MTBE-degrading propane oxidizing microorganisms and provide insight regarding propane and oxygen loading requirements and the presence of geochemical conditions that could limit microbial activity (e.g., low pH).

## A. 6 References Cited and Additional Suggested Information

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